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AN ECO-FRIENDLY APPROACH FOR ACHIEVING BETTER EXHAUSTION OF THE DYE

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ABSTRACT

In this study an try has been made to enhance the exhaustion of dye with the use of proteolytic enzyme. Dyeing is an vital method in the leather-based enterprise. it is widely recognized that some of the dyes do no longer exhaust completely and this reasons difficulty, as the bio treatability of the unexhausted sulfur dyes in effluent is generally tough. Environmental concern needs the improvement of the exhaustion of sulfur dye. To gain expanded uptake of dye, an green dyeing technique is designed the usage of enzymes. The consequences of various pH situations of enzymatic treatment on the exhaustion of the dye were studied and the circumstance optimized. The improvement in dye exhaustion is carried out the usage of extremely low amounts of enzymes within the presence of different submit tanning auxiliaries at optimized pH. The advanced exhaustion fee changed into analyzed and determined by means of spectrophotometer. stepped forward exhaustion of dyes and other post tanning chemicals will no longer most effective lessen pollutants but additionally bring about great leathers at reduced quantity of chemicals presented.

KEYWORDS: Proteolytic Enzyme, Treatability & Chemicals Presented

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Original Article

INTRODUCTION

Introduction to Enzymes

Enzymes are organic catalysts accountable for assisting nearly all of the chemical response that continues animal homeostasis. Because of their function in maintaining existence process, the assay and pharmacological law of enzymes have become key elements medical analysis and therapeutics. The macromolecular additives of virtually all enzymes are composed of protein, besides for a class of RNA modifying catalysts referred to as ribozymes. Ribozymes are molecules of ribonucleic acid that catalyze reactions on the phosphodiester bond of other RNAs.

Class of Enzymes

Traditionally, enzymes had been sincerely assigned names via the investigator who discovered enzymes then. As understanding accelerated, structures of enzyme classifications became more complete and complex. Currently enzymes are grouped into six functional lessons with the aid of the international Union of Biochemists.

Enzyme Technology Application in Leather Processing

The emphasis on the use of enzymes has come approximately due to particular residences of the enzymes. The maximum essential properties are the catalysis of chemical reactions at excessive fee under moderate environmental conditions of pH, temperature and pressure, specificity of reactions, minimum facet operations,

riskless nature and nonpolluting effluent generations. The leather industry world over is coming underneath strain from environmental guidelines to comply with the pollution and discharge legislation. The current interest inside the area of leather-based processing is moving towards the design and usage of purifier and softer technology like enzymatically improved processes. The enzymes are efficaciously employed for the higher pleasant leather production with much less pollution impact and additionally for the remedy of waste discharged from the enterprise. The leather-based processing from the raw skins to the completed merchandise required the diverse steps like curing, soaking, liming, dehairing, bathing, pickling, degreasing, tanning and dyeing. some of these successive steps in leather manufacturing contain enzymatic movement at once or in a roundabout way for facilitating the procedures and enhancing the leather output of favored best. **R B Choudhary, Indian journal of chemical technology, vol 11, September 2004)**

REVIEW OF LITERATURE

Protease Enzyme

Bacillus species are cardio, sporulating, rod-shaped bacteria which might be ubiquitous in nature. Bacillus species are used in many medical, pharmaceutical, agricultural, and business techniques that take advantage in their extensive range of physiologic characteristics and their ability to provide a host of enzymes, antibiotics, and other metabolites. Early in 1977, Priest et al., it changed into, reported that the gram-advantageous, spore forming bacterium Bacillussubtilis produces and secretes proteases, esterases, and other styles of exoenzymes at the cease of the exponential section of growth. Bacitracin and polymyxinare famous antibiotics obtained from Bacillus species. several species are used as standards in scientific and pharmaceutical assays. sure Bacillus species are important within the herbal or artificial degradation of waste products. some Bacillus insect pathogens are used as the active substances of pesticides. however many Bacillus species are being resistant to warmth, radiation, disinfectants, and desiccation, they may be difficult to put off from clinical and pharmaceutical materials and may be a cause of infection. further, Bacillus species are widely recognized inside the meals industries as difficult spoilage organisms. as a result, strategies learnt and used on this take a look at can also be implemented in fine warranty and great control departments of scientific and pharmaceutical industries in addition to in food processing The circle of relatives Bacillaceae, together with rod-shaped micro organism that shape endospores, has most important subdivisions: the anaerobic spore-forming bacteria of the genus Clostridium, and the aerobic or facultatively anaerobic spore-forming micro organism of the genus Bacillus regularly referred to as ASB(cardio spore-bearers). Bacterial cells of Bacillus cultures are Gram effective whilst younger, but in some species grow to be Gram poor as they age and therefore, it's far to be ensured that enzyme production be completed while the cultures are in exponential phase. Proteolytic enzymes are ubiquitous in prevalence, being found in all living organisms, and are critical for mobile increase and differentiation.

The extracellular proteases are business fee and discover a couple of applications in diverse commercial sectors. even though there are many microbial resources available for generating proteases, just a few are recognized as commercial manufacturers (Gupta, et al., 2002b). of these, strains of Bacillus sp. dominate the industrial area (Gupta et al., 2002a). further to that, numerous people investigated the production of protease and alkaline protease from Bacillus subtilis (Uchida et al., 1972; Daguerre et al., 1975; Remeikaite, 1979;Massucco, 1980; Gomaa et al., 1987) and explaining that only small amounts are produced by means of them and subsequently the comprehensive approach to purify and clone isolates for manufacturing and enzyme purification (Andrade et al., 2002). Proteases represent one of the most crucial organizations of business enzymes and account for as a minimum a quarter of the full global enzyme production

(Layman, 1986). distinctive species of bacteria produce acidic, neutral and alkaline proteases. The manufacturing of extra cell proteases is ruled, at the least in component, of to be had individual nutrients (North, 1982). Because microorganisms can be made to propagate hastily and profusely, they may be a super source for enzymes. (Rehm, 1986). Proteases are energetic at mild conditions, with pH optima inside the range of 6 to 8; they may be strong and strong, do no longer require stoichiometric cofactors and also are especially stereo and vicinity selective (Bordusa, 2002). those properties are quite relevant to apply them as catalysts in organic synthesis. this is viable because proteases can't best catalyze the cleavage of peptide bonds however also their formation (Capellas et al., 1997; Björup et al., 1999; So etal., 2000), in addition to other reactions of relevance for natural synthesis, forinstance: the location specific hydrolysis of esters and the kinetic decision of racemic combination, (Khmelnitsky et al., 1997; Carrea and Riva, 2000; Bordusa, 2002 Extracelluar). Subtilisin, chymotrypsin, trypsin and papain had been broadly used proteases within the chemical synthesis of peptides.

Applications of Protease

Proteolytic enzymes account for nearly 60% of the economic market within the international. They find application in a number of biotechnological lprocesses, viz. in food processing, and prescribed drugs, leather enterprise, silk, bakery, soy processing, meat tendering and brewery industries. however, itsutility in the manufacturing of peptide synthesis in natural media is confined through the presence of organic solvents. (Rahman et al., 2005).

Leather-based is tough substrate to dye due to the anisotropic nature of raw cloth. To achieve the objective of a stage and uniform dyeing with most uptake of dyes to the leather-based. The leather-based dyer wishes to be skilled and feature an intensive understanding of the dyeing houses of the dyes and auxiliaries used. Uniformly tanned leather-based, proper put up tanning treatment and additionally appropriate choice of dyes are essential for a fair colour and maximum uptakes of dyes. It have been shown that distinctive kinds of dyes have special exhaustion prices and this can lead to problems in attaining levelness and color consistency. The predominant reason for the unique dyeing behavior of dyes in leather-based dyeing is their varying affinity for the leather-based substrate and the variations the various dyes themselves. The behavior of dyes is by and large determined with the aid of the rate characteristics of both the dye and the leather-based to be dyed. however, use of suitable auxiliaries can minimize the differences in dyeing conduct among the dye and the leather-based.

Variations in exhaustion fee or bathtub exhaustion and boom inside the dye content on particular leathers are the precept issues. In most of these instances, a stability must be obtained between the dyeing conditions, post tanning chemical substances and the auxiliaries used for dyeing (studies on the have an impact on at the proteolytic enzymes in leather-based dyeing S. V. Kanth, Jalca 2006).

OBJECTIVE

To improve the exhaustion of dye by using extremely low amount of enzyme in the presence of other post tanning auxiliaries at optimized pH.

MATERIALS AND METHODS

Methods

Isolation and Extraction of Enzyme

Nutrient broth medium (g/l)

Nutrient agar media (g/l)

Sub Culturing

The mother culture AU1, AU2, AU3, AU4, AU5 was used for sub culturing. They were streaked in the nutrient agar medium and then kept for incubation at 37^0Cfor 24 hours.

Production of Enzyme

Nutrient broth was used for proteolytic enzyme production. A quantity of 250ml of this broth was dispensed in 500ml of Erlenmayer flask and sterilized at 1atm for 1hour in autoclave and inoculated with AU3 sub culture. It was then incubated at 37^0Cfor 48hours in incubating shaker at 75rpm. Then the broth was centrifuged at 12000rpm for 30mins at 4c and the supernatant was taken for the experiment. The supernatant containing the crude enzyme was purified and assayed for activity.

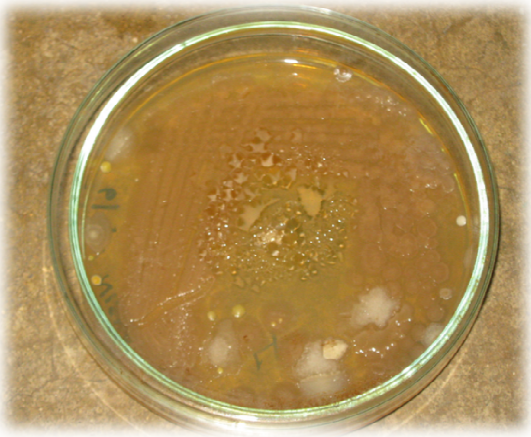

Figure 1: Subculture of Bacillus Subtilus

Enzyme Assay

For protease assay, the method adopted by kunitz (1947) was modified and used.

Purification of Enzyme

Ammonium sulphate protein precipitation is a usually used technique for purifying proteins. Protein precipitate at unique concentrations of ammonium sulphate relative to their internet charge density.

Dialysis

Dialysis to remove salts - the precept of dialysis is that semi-permeable membranes exist with sure sized holes in them which permit small molecules to pass across the membrane.

Figure 2: Dialysis of Crude Enzyme

Calculation for Enzyme Assay

Crude Enzyme Activity

Sample OD: 0.444

Corresponding concentration: 0.12 µmole Tyr/ml

1µ mole/ml Tyr ⟶ 181 µg/ml

0.12µmole/ml Tyr ⟶ 0.12×181

=21.72µg/ml Tyr

Dialysed Enzyme Activity

Sample OD: 0.613

Corresponding Concentration: 0.15 µmole Tyr/ml

1µ mole/ml Tyr ⟶ 181 µg/ml

0.15µmole/ml Tyr ⟶ 0.15×181

=27.15µg/ml Tyr

Table 1: Experimental Post Tanning Process

Process	%	Chemicals	Duration	pH Adjustments
Washing	200%	Water	15 min	
Neutralization	100% 1% 0.5%	Water Sodium formate Sodium bicarbonate	20 min 60 min	pH 5 pH 5.5
Retanning	100% 2%	Water Relugan RE Sodium bicarbonate	20 min 30 min	pH 5.5, 5.8,6
Enzyme treatment (varying pH)	1%	Alkaline protease	30 min	pH 5.5, 5.8,6
Dyeing / fatliquoring	2.5%	Dye	30 min	
	2%	Synthetic fat liquor	15 min	
	2%	Phenolic syntan	15 min	
	5%	Synthetic fat liquor	10 min	

	2%	Semisynthetic fat liquor		
	0.1%	Preservative	45 min	
	4% 4%	Phenolic syntan Melamine HCHO syntan	45 min	
	2.5% 10%	Formic acid water	3×10 min+ 30 min	

RESULTS AND DISCUSSIONS

Enzyme Activity Analysis

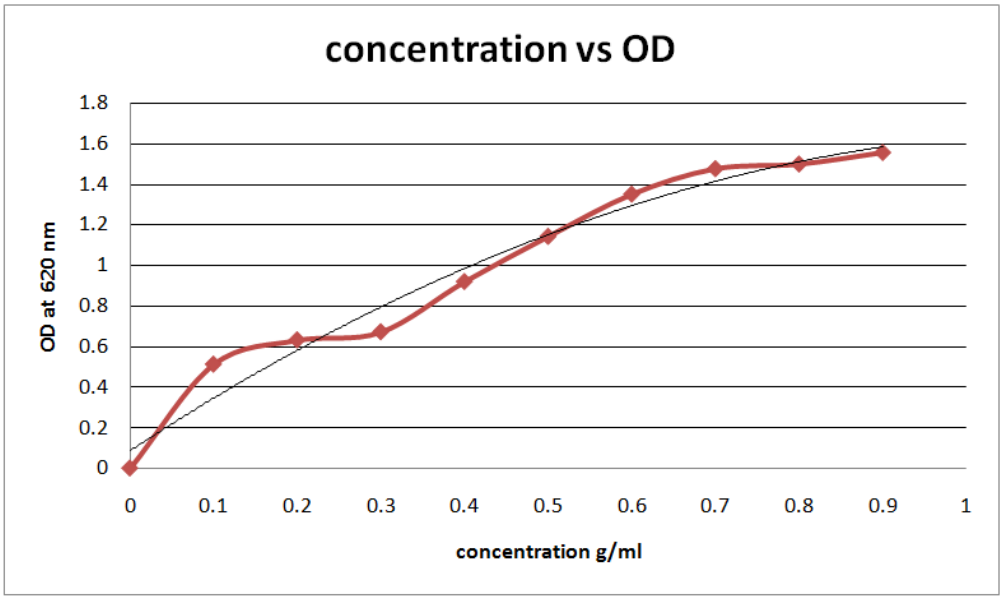

Figure 3: Protease Enzyme Assay

Enzyme Applications for Dye Exhaustion

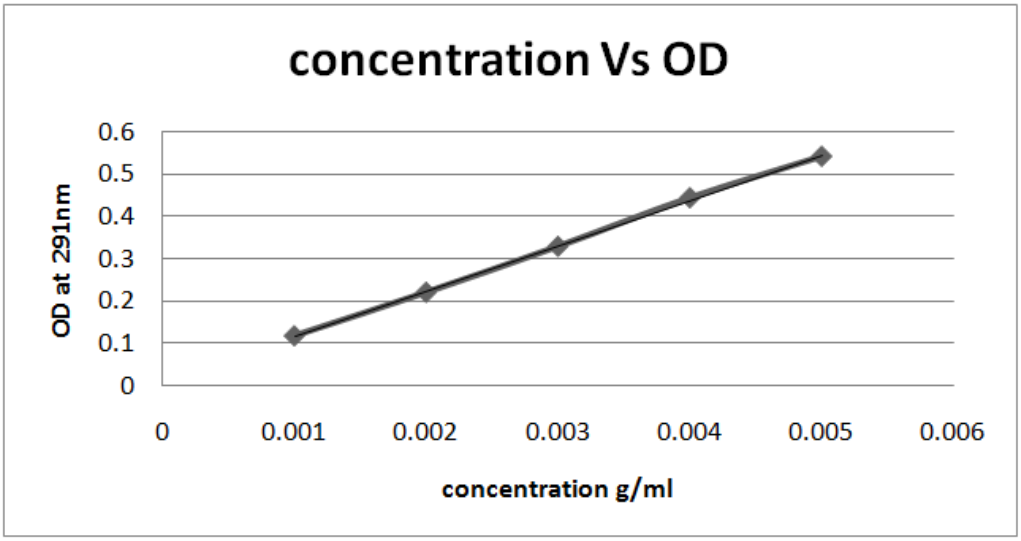

Figure 4: Standard Graph for Chocolate Brown Dye

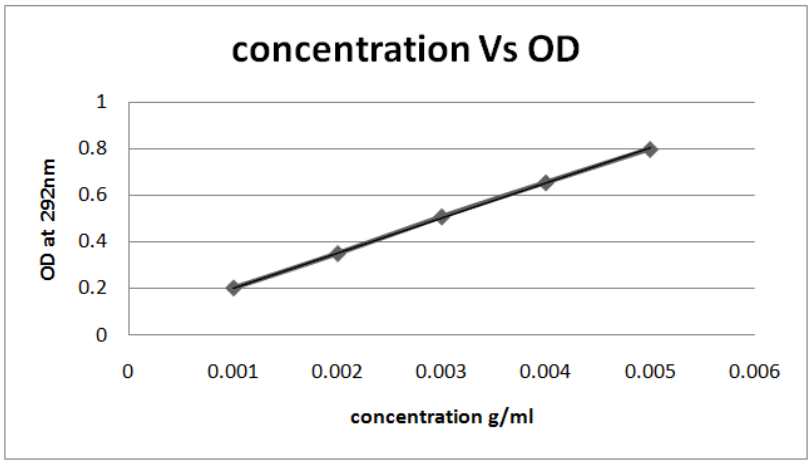

Figure 5: Standard Graph for Tancol Black Dye

CONCLUSIONS

Enzymes have been used as an eco-friendly approach for achieving better exhaustion of the dye. The method is primarily based on the concept that the enzymes act as biocatalysts in establishing up the fibrous leather community, which increase the diffusion of the dyes into the leather matrix and also the contact surface areas in the leather exposed for interaction with the dye increases. One percent offer of enzyme, at pH of 5.8 for 30 min is found to be optimum with respect to the uptake of the dye. Enzyme pretreatment at this optimized condition has resulted in good opening up and splitting of fiber structure resulting in 94% exhaustion of tancol black sulfur dye LSG, whereas 95.2% exhaustion of chocolate brown LFP. Thus poorly exhausting sulfur dyes has been improved by enzymatic treatment and good exhaustion is obtained.

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